# Spatial effects of retention trees on mycorrhizas and biomass of Douglas-fir seedlings

**E. Cline, B. Vinyard, and R. Edmonds**

**Abstract:** Retention forestry places seedlings in proximity to residual trees, exposing seedlings to additional sources of ectomycorrhizal fungus (EMF) inoculum. To investigate this, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings were planted near (2–6 m) and far (16–30 m) from 44- to 72-year-old residual Douglas-fir trees in western Washington, USA. From 1998 through 2000, seedling shoot and root biomass was assessed and EMF taxa were identified using morphology and sequence analysis of internal transcribed spacer and large subunit ribosomal RNA genes. Seedlings near residual trees had significantly greater ectomycorrhiza (ECM) abundance (percent active ECM root tips), less necrotic root tips, and higher root to shoot biomass ratios. Seedlings near trees had a richness index of 4.1 EMF taxa per seedling and 42 total taxa compared with 3.5 taxa per seedling and 33 total taxa for seedlings far from trees. Proximity to residual trees may increase seedling ECM abundance and diversity.

**Résumé :** Dans le cadre de la foresterie avec rétention, les semis sont plantés près des arbres résiduels, ce qui les expose à des sources additionnelles d'inoculum de champignons ectomycorhiziens (CEM). Pour étudier cette pratique, des semis de douglas vert (*Pseudotsuga menziesii* (Mirb.) Franco) ont été plantés près (2–6 m) et loin (16–30 m) de douglas vert âgés de 44 à 72 ans dans l'ouest de l'État de Washington, aux États-Unis. De 1998 à 2000, la biomasse aérienne et souterraine des semis a été évaluée et les taxons de CEM ont été identifiés à l'aide de critères morphologiques et du séquençage des espaceurs transcrits internes et des gènes formés d'importantes sous-unités d'ARN ribosomique. Les semis situés près des arbres résiduels étaient caractérisés par une abondance d'ectomycorhizes (ECM) significativement plus grande (pourcentage d'apex racinaires colonisés par des ECM actives), moins d'apex racinaires nécrosés et un rapport de la biomasse des racines sur la biomasse de la tige plus élevé. Les semis situés près des arbres avaient un indice de richesse de 4,1 taxons de CEM par semis et un total de 42 taxons comparativement à 3,5 taxons par semis et un total de 33 taxons pour les semis situés loin des arbres. La proximité des arbres résiduels peut augmenter l'abondance et la diversité des ECM sur les semis.

[Traduit par la Rédaction]

## Introduction

Management strategies involving retention forestry have been proposed as an alternative to clear-cutting of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) forests in the Pacific Northwest (Kohm and Franklin 1997). While patterns and densities of residual trees can vary, retention forestry (or "green-tree retention") refers to harvesting in which some proportion of the living trees are protected. Residual trees can lessen the impacts of harvesting by reducing erosion, moderating the effects of wind and solar exposure, and harboring organisms that would otherwise be excluded by clear-cutting (Perry et al. 1989; North et al. 1996). Mounting concern over the impacts of clear-cutting has led to widespread adoption of provisions for retention of green trees, snags, and logs by private (WFPA 1995) and public (Forest Ecosystem Management Assessment Team 1993; WFPB 1995) forest managers throughout the Pacific Northwest. However, more testing is needed to determine the impacts and benefits of retention forestry.

In the western foothills of the Cascade mountain range, Douglas-fir is considered to be shade-intolerant and unable to regenerate under a closed canopy (Franklin and Dyrness 1988). Survival and growth of Douglas-fir seedlings after partial retention harvesting may depend on the density of residual trees. In mature, thinned Douglas-fir stands in western Oregon, planted Douglas-fir seedlings had low survival rates (Brandeis et al. 2001). However, Edmonds et al. (2000) found no significant difference in Douglas-fir seedling survival over the first growing season in seedlings planted from 1 m to 6 m from retention trees in western Washington. While seedlings near trees would be expected to experience increased competition, residual trees might also facilitate seedling survival and growth. Root systems could enhance seedling colonization by mycorrhizal fungi, helping the regenerating stand to retain a functionally diverse array of mycorrhizal fungi.

A number of studies have documented differences in the mycorrhiza status of seedlings planted within the rooting zones of residual trees or of forest edges. Dickie et al.

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(2002) demonstrated that red oak (*Quercus rubra* L.) seedlings near harvested, stump-sprouting mature oak trees had greater mycorrhiza colonization and diversity, which they attributed to a "nurse tree" effect. In northwest British Columbia, paper birch (*Betula papyrifera* Marshall) seedlings had higher mycorrhiza diversity when growing near mature paper birch trees both in clearcuts and in mixed conifer forests (Kranabetter and Wylie 1998). In the same region, planted western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and lodgepole pine (*Pinus contorta* Douglas ex Loudon) seedlings (Durall et al. 1999), and naturally regenerated western hemlock seedlings (Kranabetter and Wylie 1998) near the mature forest edge, had higher ectomycorrhizal fungus (EMF) diversity than seedlings located further into the forest opening. When naturally regenerated western hemlock seedlings from the forest were transplanted into clearcuts, mycorrhiza diversity declined (Kranabetter and Friesen 2002). In clearcuts in the southern interior of British Columbia, Hagerman et al. (1999) showed that hybrid Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) × white spruce (*Picea glauca* (Moench) Voss) bioassay seedlings planted 2 m from forest edges had higher EMF species richness than seedlings planted 16 m into clearcuts, although proximity to the forest edge had no effect on previously colonized Engelmann spruce seedlings planted in mineral soil exposed by mechanical mounding (Jones et al. 2002). Ectomycorrhizal fungus species richness of planted western hemlock and lodgepole pine seedlings decreased beyond 7 m distance from the forest edge into clearcuts (Durall et al. 1999). These studies provide support for the hypothesis that residual trees could enhance mycorrhiza colonization of seedlings, although Jones et al. (2003) point out that shifts in the EMF community in clearcuts could be adaptive, in response to changes in the soil chemistry, biology, or other aspects of the physical environment.

The effect of proximity to residual trees on Douglas-fir seedlings has not been well investigated in the Pacific Northwest, although retention forestry has recently been widely adopted in management of western Douglas-fir forests (Forest Ecosystem Management Assessment Team 1993; WFPB 1995). Because maintenance of biodiversity has increasingly become a priority in management of both public and private forest lands (Kohm and Franklin 1997), it is necessary to evaluate the effectiveness of partial canopy retention for promoting diversity of EMF communities in regenerating stands.

The objective of this study was to assess the spatial influence of residual trees on the ectomycorrhiza (ECM) of Douglas-fir seedlings. We hypothesized that seedlings near residual trees would have (*i*) greater levels of EMF colonization, and (*ii*) higher numbers of EMF taxa per seedling than seedlings far from trees.

## Materials and methods

### Study sites and characteristics

The three study sites were located in the western foothills of the Cascade mountain range in Washington State approximately 50 km southwest of the city of Seattle. The sites were between 7 and 13 km apart from one another. Two sites, "Beatles" (47°20.970′N, 121°49.895′W) and "Imagine" (47°23.956′N, 121°48.938′W), were in the Cedar River watershed, managed by the city of Seattle, while one site, "Green River" (47°18.719′N, 121°42.609′W), was located in the adjacent Green River watershed on land owned and managed by the Plum Creek Timber Company. The Green River site was harvested only 2 years before the study began, while Imagine was harvested 4 years and Beatles 6 years before the study commenced in 1998.

The Green River site was different from the other two sites in several important respects. The forest was younger at the time of harvest at the Green River site, reflected by the age of the residual tree of 44 years versus 72 and 69 years at Imagine and Beatles, respectively (Table 1). The residual trees at Imagine and Beatles were consequently both taller and larger in diameter than the Green River residual tree, but were similar in age and size to one another.

Vegetation at the sites was typical of low-elevation forest clearcuts and early successional forests. In addition to residual Douglas-fir trees and planted seedlings, other conifer species at the sites included young and mature western hemlock, Pacific silver fir (*Abies amabilis* (Dougl.) Forbes), western redcedar (*Thuja plicata* (L.) Donn.), and grand fir (*Abies grandis* (Dougl.) Forbes). Vegetation and other site attributes have been described elsewhere (Cline 2004; Cline et al. 2005).

### Sampling design and field measurements

One Douglas-fir tree that had been retained during harvesting was selected to be the center tree at each of the three sites. In an earlier study at the same sites, we had determined that the roots of each center tree extended no more than 8 m from the base of the tree (Cline 2004). In April 1998, mycorrhizal nursery-grown 2-1 (3-year-old) Douglas-fir seedlings were planted within the area defined by concentric circles 2–6 m from the base of the center tree and designated as "2–6 m" seedlings. The "16–30 m" seedlings were planted from 16 to 30 m from the center tree. Buried, thoroughly decayed soil wood was common at all three study sites. The predominant soil substrate for each seedling was noted as either mineral soil or buried soil wood. Equal numbers of seedlings were planted into each soil substrate type. Seedlings grown under standard nursery practices were used, which were mycorrhizal at the time of planting. This planting method closely mimics normal private and public forestry practices. At each center tree, forty 2–6 m and forty 16–30 m seedlings were planted at a spacing of at least 1 m from one another and from previously established Douglas-fir seedlings at the site. Where necessary, understory vegetation was cleared to provide space for the planted seedlings. Root volumes were determined by water displacement for all seedlings before planting. Seedlings were immersed in water in a beaker equipped with an overflow spigot, and the volume (mL) of displaced water was measured with a graduated cylinder and converted to $cm^3$.

Ectomycorrhizal fungus taxa on seedling roots were sampled in October 1998, 6 months after planting (MAP), June 1999 (12 MAP), October 1999 (18 MAP), and June 2000 (27 MAP) with 100 roots from each of 8 seedlings for each of the proximity groups at each of the three sites for the first three samplings. The final sampling was limited to 4 seedlings for each treatment group at each site, owing to loss of seedlings to mountain beaver herbivory at the Green

**Table 1.** Site harvest year, elevation, and Douglas-fir residual tree attributes.

| | | | Residual tree attribute | | |
|---|---|---|---|---|---|
| | Harvest year | Elevation (m) | Age (years) | Height (m) | DBH (cm) |
| Green River | 1996 | 808 | 44 | 34 | 43.4 |
| Imagine | 1994 | 457 | 72 | 44 | 66.3 |
| Beatles | 1992 | 488 | 69 | 42 | 62.5 |

River site. Seedlings were excavated, transported back to the laboratory at the University of Washington, and stored at 4 °C. After removing mycorrhizal root tips for analysis, the subsampled feeder roots were combined with the remaining root system for biomass measurement. Roots and shoots were dried at 45 °C to constant weight to determine the root and shoot biomass (g) for each seedling. Root biomass was determined at 6, 18, and 27 MAP, while shoot biomass was determined at only 18 and 27 MAP. We did not measure shoot biomass at 6 MAP; therefore, this value was estimated based on stem volume calculated from seedling height and diameter. For each growing season, seedling height (cm) was measured to the base of the apical bud and root collar diameter (cm) was measured with calipers.

### Analysis of seedling ECM

Seedlings were kept refrigerated with roots attached to the shoot for a period not exceeding 4 weeks before mycorrhiza analysis. The root system was gently washed to remove excess soil, then feeder roots were subsampled, excised and immersed in water in a shallow tray over a 1 cm square grid and examined under an 8–40× dissecting microscope. At each of the 100 grid points, the nearest root tip was sorted into preliminary categories of inactive or necrotic, non-mycorrhizal, and mycorrhizal. Root tips lacking a visible mantle were presumed to be mycorrhizal and sampled for molecular analysis unless abundant root hairs were present. Root tips were considered inactive or necrotic if the entire root tip was shrunken and dessicated. Mycorrhizal root tips were sorted into broadly defined morphotypes based on morphological characteristics including branching structure and shape, mantle color and texture, and emanating hyphae and rhizomorphs based on described methods (Ingleby et al. 1990; Agerer 1991; Goodman et al. 2000). Root tips from each morphotype for each seedling were counted and lyophilized to prepare for long-term storage and (or) DNA extraction. In total, 16 800 root tips were examined during the course of this study, of which 8410 were active mycorrhizal root tips. Extraction of DNA from a subsample of root tips of each morphotype was performed using the method of Gardes and Bruns (1993), with modifications as described (Cline 2004; Cline et al. 2005). Extracted DNA samples were stored at –40 °C for future analysis.

Restriction fragment length polymorphism (RFLP) analysis of the internal transcribed spacers (ITS) of the ribosomal RNA genes was used to distinguish among EMF taxa, while identification was accomplished by determining the sequence of the nuclear ITS or large subunit (nLSU) rRNA genes for each taxon, as described (Cline et al. 2005). RFLP patterns were analyzed for a total of 632 root tips. Sequences of ITS and nLSU were determined for a subsample of root tips from each unique RFLP pattern, generating a total of 148 ITS and 53 nLSU sequences, an average of just over four sequences for each of the 47 EMF taxa distinguished in this study. Sequence homologies in BLAST searches (Altschul et al. 1997) of 98% or greater for the ITS region and 99% or greater for the nLSU rRNA gene were considered sufficient to assign tentative species-level designations to unidentified fungal taxa. Selected sequences from this study have been deposited in GenBank under the accession Nos. AY356323, AY750156–AY750169, and AY751555–AY751568.

### Calculation of root and shoot biomass ratios and EMF relative abundance

Shoot biomass was not measured at 6 MAP but was estimated as

$$[1] \quad \ln(1 + \text{shoot biomass}) = 1.020 \times \ln(1 + \text{stem volume})$$

This was derived from the relationship between stem volume and shoot biomass for seedlings measured at 18 and 27 MAP, using linear regression with the intercept set to zero ($F_{(1, 65)} = 20885$, $p = 0.000$, $R^2 = 0.997$) with a natural log transformation of both variables to achieve equal variance of the residuals. Stem volume was calculated following van den Driessche (1992) using the allometric equations

$$[2] \quad \text{Stem volume} = (\pi \times \text{diameter}^2 \times \text{height})/12$$

where diameter and height were measured in cm. Root to shoot ratios were calculated as the root biomass (g) divided by the shoot biomass (g). Relative abundance of each EMF taxon identified by RFLP and sequence analysis was calculated as the number of root tips with that taxon divided by the number of mycorrhizal tips, on a seedling by seedling basis.

### Statistical analyses

The 3 sites were the replicates ($N = 3$) for the study. At each site, the 2 "regions of proximity" (defined by the areas between the concentric circles at 2 m and 6 m and between 16 m and 30 m from the site's residual tree) were each a "sub-plot" of the site. From each of the 3 sites × 2 proximity regions (i.e., 6 sub-plots), seedling growth and fungal population characteristics were measured on 8 seedlings at 6, 18, and 4 seedlings at 27 MAP. Measurements on the individual seedlings were averaged to obtain a single value for each site × proximity region × MAP for use in the strip-split-plot or repeated measures ANOVA with $N = 3$ replicates (i.e., sites); a total of 3 sites × 2 proximity regions × 3 MAPs = 18 data values. The ANOVA is considered a repeated measures ANOVA because a site is repeatedly measured by sampling seedlings at 3 MAPs from the 2 proximity regions at a site (i.e., experimental unit). Similarly, the strip-split-plot label describes how "near" and "far" proximity regions (i.e., strip-plots ≡ sub-plots that cannot be randomized) at a site are further sub-sampled (i.e., split) to measure additional seedlings to test for any effect of MAP. A repeated measures ANOVA allows any correlation that may exist among seedlings sampled at different MAPs and (or) between near and far proximity regions to be modeled. The

general ANOVA table for this study was Treatment, df = 2; Error Term for Treatment = Site(Treatment), df = 4; MAP, df = 2; Treatment × MAP, df = 2; Error = Site × MAP(-Treatment), df = 8; to yield total df = 17. The characteristics examined were shoot biomass, root biomass, ratio of root to shoot biomass, percent active ECM, percent necrotic, percent non-ECM, and EMF richness index.

For each characteristic, a repeated measures ANOVA was conducted with treatment (i.e., proximity region) and MAP as the two factors, using SAS® v9.1.3 Proc MIXED. Any potential correlation between proximity regions was examined by modeling the candidate covariance structures: independent, compound symmetric, and heterogeneous compound symmetric. Similarly, potential correlations among the data values observed on seedlings from the same site across subsequent MAPs (i.e., repeated time measurements) were examined by modeling the candidate covariance structures: unstructured, independent, compound symmetric, heterogeneous compound symmetric, spatial exponential, or first-order ante-dependent. To stabilize within-treatment variances across MAPs, ANOVA was conducted on $\log_{10}$(Root Biomass) – $\log_{10}$(Shoot Biomass) values instead of ratio of root to shoot biomass. Least significant difference (LSD) means comparisons with letter groupings ($\alpha = 0.05$) were obtained using the pdmix800 SAS macro (Saxton 1998).

## Results

### Mycorrhizas of seedlings

Between 6 and 27 MAP, a total of 5600 root tips from 56 seedlings were examined at each site, of which 2876 root tips had active mycorrhizas at Green River, 2817 at Imagine, and 2717 at Beatles. Root tips with root hairs but no visible mantle could have been colonized by endomycorrhizal fungi (Cazares and Smith 1996); however, no endomycorrhizal fungus taxa were detected through molecular methods on root tips colonized by ecto- (or ectendo-) mycorrhizal fungi in this study. A total of 47 distinguishable EMF taxa were observed on seedlings at the three study sites (Table 2). Of those, 26 taxa were found at Green River, 30 at Imagine, and 35 at Beatles.

The proportion of root tips that were ectomycorrhizal (percent active ECM) from 6 to 27 MAP averaged 55.7% of total tips for 2–6 m seedlings and 43.6% for 16–30 m seedlings. A substantial proportion of root tips were designated as necrotic. Most necrotic tips appeared to have been colonized by mycorrhizal fungi, but they were dessicated or decayed such that identification of the EMF taxon was no longer possible, and the root tip appeared to be inactive. Non-mycorrhizal root tips, distinguished by abundant root hairs, consisted predominantly of newly formed branch roots located near the growing tip of the root. For 2–6 m seedlings, an average of 38.5% of root tips were necrotic and 5.8% were non-mycorrhizal, while seedlings 16–30 m from residual trees had 49.8% necrotic and 6.6% non-mycorrhizal root tips.

### Effects of proximity to residual trees on seedling mycorrhizas

The proportion of root tips with active mycorrhizas was assessed for seedlings planted 2–6 m and 16–30 m from residual trees at 6, 18, and 27 MAP. On average, seedlings growing near residual trees had a higher percent active ECM throughout the study period (Fig. 1*a*), with the strongest treatment effect at 18 MAP. The treatment effect was significant, based on multi-factor repeated measures ANOVA with a variance component (i.e., independence) covariance structure among MAPs and between treatments ($F = 16.8$, $p = 0.0015$), while MAP was not a significant factor. Hereafter, only non-independent (i.e., not variance component) covariance structures among MAPs or between treatments will be reported. The proportion of necrotic root tips was positively related to MAP ($F = 10.2$, $p = 0.003$) in repeated measures ANOVA. On average, 2–6 m seedlings had a smaller proportion of necrotic root tips (Fig. 1*b*); the treatment effect was significant ($F = 17.9$, $p = 0.001$). There was no significant treatment effect on the proportion of non-ECM root tips ($F = 0.51$, $p = 0.5$). Instead, the percent non-ECM was negatively related to MAP, decreasing dramatically over the period of the study ($F = 128$, $p = 0.0004$) in repeated measures ANOVA with covariance structure unstructured among-MAPs (Fig. 2*a*).

On average, 16–30 m seedlings had 3.5 EMF taxa, while 2–6 m seedlings had an average of 4.1 taxa. The treatment group was not significantly related to the EMF richness index, based on multi-factor repeated measures ANOVA with a first-order ante-dependent covariance structure (Fig. 1*c*). The EMF richness index was significantly related to MAP ($F = 35.8$, $p = 0.0038$).

### Seedling biomass

Root biomass increased from 11.6 ± 0.4 g before planting to 28.0 ± 2.1 g (2.4-fold) for 2–6 m seedlings and 29.1 ± 2.4 g (2.5-fold) for 16–30 m seedlings at 6 MAP (Table 3) with between-treatment compound symmetric and among-MAPs spatial exponential covariance structures. This rapid root growth was reflected by an increase in root to shoot biomass ratios from 0.66 ± 0.03 before planting to 0.95 ± 0.06 for 2–6 m seedlings and 0.85 ± 0.06 for 16–30 m seedlings at 6 MAP (Fig. 2*b*). The root to shoot biomass ratio was greater for 2–6 m seedlings for each MAP, although there was no significant difference by LSD ($\alpha = 0.05$) when each MAP was considered individually (Fig. 2*b*). Nevertheless, the treatment effect on log transformed root to shoot ratio was significant based on multifactor repeated measures ANOVA ($F = 7.77$, $p = 0.0164$), with MAP also a significant factor ($F = 78.4$, $p < 0.0001$).

By 27 MAP, 2–6 m seedlings had a somewhat greater average root biomass, while shoot biomass was similar to that of 16–30 m seedlings (Table 3). MAP was a significant factor both for shoot biomass ($F = 50.0$, $p = 0.001$) with heterogeneous compound symmetric among-MAPs covariance structure and root biomass ($F = 6.3$, $p = 0.022$), but no significant relationship was detected between seedling treatment group and shoot biomass or root biomass by multifactor repeated measures ANOVA.

## Discussion

### Mycorrhizas of seedlings

The total of 47 EMF taxa observed on Douglas-fir seedlings in this study is greater than the 33 morphotypes ob-

**Table 2.** Most similar BLAST sequences and relative abundance (%) for EMF taxa on Douglas-fir seedlings.

| Most similar BLAST sequence | rRNA type | % similarity | Accession No. | Source | Consensus EMF taxon | 2–6 m seedlings[a] | 16–30 m seedlings[b] |
|---|---|---|---|---|---|---|---|
| *Russula nigricans* (Bull.) Fr. | ITS | 99 | AF418607 | BLAST | *Russula nigricans* | 14.9 | 0.1 |
| *Rhizopogon rudus* A.H. Sm. | ITS | 98 | AF377107 | BLAST | *Rhizopogon rudus* | 11.7 | 23.9 |
| *Rhizopogon vinicolor* A.H. Sm. | ITS | 98 | AF263933 | BLAST | *Rhizopogon vinicolor* I | 10.4 | 13.4 |
| *Pseudotomentella tristis* (P. Karst.) M. Larsen | ITS | 99 | AF274772 | BLAST | *Pseudotomentella tristis* | 9.3 | 11.0 |
| *Boletus zelleri* Murrill | ITS | 100 | AY750158 | ec176.c41[c] | *Boletus zelleri* | 8.6 | 3.2 |
| *Tuber borchii* Vittad. | ITS | 94 | AF106890 | BLAST | *Tuber* I | 6.5 | 10.6 |
| *Rhizopogon parksii* A.H. Sm. | ITS | 100 | AF058314 | BLAST | *Rhizopogon parksii*[e] | 6.4 | 7.3 |
| *Tomentella sublilacina* (Ellis & Hollw.) Wakef. | ITS | 99 | AF323111 | BLAST | *Tomentella sublilacina* | 3.9 | 1.2 |
| *Tomentella stuposa* (Link) Stalpers | ITS | 99 | AY010277 | BLAST | *Tomentella stuposa* | 2.9 | 1.6 |
| *Cenococcum geophilum* Fr. | ITS | 99 | AY112935 | BLAST | *Cenococcum geophilum* | 2.6 | 5.6 |
| *Tomentella ellisii* (Sacc.) Jülich & Stalpers | ITS | 94 | AF272913 | BLAST | *Tomentella ellisii* gp. | 2.4 | 2.9 |
| *Melanogaster macrosporus* Velen. | ITS | 96 | AJ555526 | BLAST | *Melanogaster* I | 2.4 | 0.3 |
| *Tylospora asterophora* (Bonord.) Donk | LSU | 96 | AY463480 | BLAST | *Tylospora* I | 2.3 | 1.1 |
| *Russula bicolor* Burl. | ITS | 99 | AY750161 | ec179.c83[c] | *Russula bicolor* | 1.9 | |
| *Russula chloroides* (Krombh.) Bres. | ITS | 98 | AF418604 | BLAST | *Russula chloroides* | 1.6 | |
| *Clavulina cristata* (Holmsk.) J. Schröt. | LSU | 99 | AY586648 | BLAST | *Clavulina cristata* | 1.4 | 2.1 |
| *Tylospora asterophora* (Bonord.) Donk | LSU | 100 | AF325323 | BLAST | *Tylospora asterophora* | 1.2 | 0.4 |
| *Lactarius mitissimus* (Fr.) Fr. | ITS | 97 | AF157412 | BLAST | *Lactarius* I | 1.2 | |
| *Laccaria laccata* (Scop.) Fr. | ITS | 95 | AF204814 | BLAST | *Laccaria* I | 1.0 | |
| *Truncocolumella citrina* Zeller | ITS | 98 | L54097 | BLAST | *Truncocolumella citrina* | 0.9 | |
| *Thelephora terrestris* Ehrh. | ITS | 99 | AY750163 | ec181.c128[c] | *Thelephora terrestris* | 0.8 | 3.7 |
| *Amanita muscaria* (L.) Hook | LSU | 99 | AF097367 | BLAST | *Amanita muscaria* | 0.7 | |
| *Sebacina sp.* | LSU | 99 | AF440647 | BLAST | *Sebacina* I | 0.7 | |
| *Hebeloma cavipes* Huijsman | ITS | 99 | AF124670 | BLAST | *Hebeloma cavipes* | 0.6 | 1.0 |
| *Wilcoxina rehmii* Chin S. Yang | ITS | 99 | AF266708 | BLAST | *Wilcoxina rehmii* | 0.6 | 0.2 |
| *Tomentella lateritia* Pat. | ITS | 93[d] | AJ534912 | BLAST | *Tomentella* II | 0.5 | 0.4 |
| *Pseudotomentella nigra* (Höhn. & Litsch.) Svrček | ITS | 99[d] | AF274770 | BLAST | *Pseudotomentella nigra* | 0.5 | 1.1 |
| *Inocybe sindonia* (Fr.) P. Karst | LSU | 96 | AY380393 | BLAST | *Inocybe sindonia* gp. | 0.2 | |
| *Russula xerampelina gp.* (Schaeff.) Fr. | ITS | 100 | AY750164 | ec182.c130[c] | *Russula xerampelina* gp. | 0.2 | |
| *Peziza limnaea* Maas Geest. | LSU | 99 | AF335147 | BLAST | *Peziza limnea* | 0.2 | |
| *Piloderma fallax* (Lib.) Stalpers | ITS | 99 | AY010281 | BLAST | *Piloderma fallax* | 0.2 | 0.3 |
| *Piloderma byssinum* (P. Karst.) Jülich | ITS | 98 | AY010279 | BLAST | *Piloderma byssinum* | 0.2 | |
| *Inocybe pudica* Kühner | LSU | 94 | AY038323 | BLAST | *Inocybe* II | 0.1 | 0.3 |
| *Amphinema byssoides* (Pers.) J. Erikss. | LSU | 96 | AY568626 | BLAST | Atheliaceae I | 0.1 | 1.5 |
| *Macowanites americanus* Singer & A.H. Sm. | ITS | 100 | | S.L. Miller | *Macowanites americanus* | 0.1 | |
| *Athelia neuhoffii* (Bres.) Donk | ITS | 89[d] | U85798 | BLAST | Atheliaceae II | 0.1 | 0.5 |
| *Russula sphagnophila* Kauffman | LSU | 98 | AF506464 | BLAST | *Russula sphagnophila* gp. | 0.1 | 0.3 |
| *Dermocybe cinnamomea* (L.) M.M. Moser | ITS | 99 | AY750159 | ec177.c56[c] | *Dermocybe cinnamomea* | 0.1 | 0.1 |

**Table 2** (*concluded*).

| Most similar BLAST sequence | rRNA type | % similarity | Accession No. | Source | Consensus EMF taxon | 2–6 m seedlings[a] | 16–30 m seedlings[b] |
|---|---|---|---|---|---|---|---|
| *Amphinema byssoides* (Pers.) J. Erikss. | LSU | 99 | AF291288 | BLAST | *Amphinema byssoides* | 0.1 | 0.1 |
| *Inocybe praetervisa* Quél. | LSU | 97 | AY038322 | BLAST | *Inocybe praetervisa* gp. | 0.1 | |
| *Inocybe flocculosa* (Berk.) Sacc. | LSU | 96 | AY380375 | BLAST | *Inocybe* I | 0.1 | |
| *Amphinema byssoides* (Pers.) J. Erikss. | LSU | 97 | AF291288 | BLAST | *Amphinema* I | 0.1 | 0.4 |
| *Phialophora finlandica* C.J.K. Wang H.E. Wilcox | ITS | 100 | AJ534704 | BLAST | *Phialophora finlandica* | | 0.7 |
| *Rhizopogon villosulus* Zeller | LSU | 100 | AF071464 | BLAST | *Rhizopogon villosulus* | | 4.1 |
| *Tomentellopsis sp.* | ITS | 95 | AJ410774 | BLAST | *Tomentellopsis* I | | 0.4 |
| *Tomentella botryoides* (Schwein.) Bourdot & Galzin | LSU | 98 | AY586717 | BLAST | *Tomentella* I | | 0.2 |
| *Inocybe sierraensis* Kropp & Matheny | LSU | 97 | AY239025 | BLAST | *Inocybe sierraensis* gp. | | 0.1 |

**Note:** Relative abundance values are mean percent of total mycorrhizal root tips per seedling. For each seedling, a subsample of 100 root tips was examined. Relative abundance value columns sum to 100%. Seedlings were planted in spring 1998. ITS, internal transcribed spacer; LSU, large subunit.

[a]2–6 m from mature trees and sampled spring and fall, from 6 to 27 MAP.

[b]16–30 m from mature trees and sampled spring and fall, from 6 to 27 MAP.

[c]Sporocarp collected at site.

[d]Partial sequence only.

[e]Could not be distinguished via RFLP from *R. vinicolor* II.

served by Roth and Berch (1992) on Douglas-fir seedlings 1 year after planting in clearcuts on Vancouver Island in Canada, but falls within the range of total numbers of morphotypes reported for other conifer seedlings. For example, in a series of studies in British Columbia, Canada, Kranabetter and colleagues reported 38 morphotypes on western hemlock seedlings (Kranabetter and Wylie 1998; Kranabetter et al. 1999; Kranabetter and Friesen 2002), and an average of 52 morphotypes per tree species for white spruce, subalpine fir, and lodgepole pine seedlings (Kranabetter et al. 1999).

We previously reported differences between the EMF communities of seedlings and mature Douglas-fir trees, and between seedlings 2–6 and 16–30 m from residual trees (Cline et al. 2005). Here we focus upon effects of proximity to residual trees on seedling-level attributes including EMF colonization levels and numbers of EMF taxa per seedling. These variables reflect the status of individual seedlings rather than the EMF community as a whole.

We observed a high proportion of necrotic root tips in this study. Many mycorrhiza studies do not directly measure the proportion of necrotic root tips, but in this case it revealed an intriguing pattern, with seedlings 2–6 m from residual trees exhibiting significantly smaller proportions of necrotic root tips. Proportions of non-ECM root tips were also relatively high in the current study, compared with values reported for other conifer seedlings, which are generally below five percent (e.g., Jonsson et al. 1999; Jones et al. 2002). Seedlings experienced rapid root growth in the first six months after planting, possibly resulting in an unusually high proportion of root tips that had not yet been colonized at 6 MAP. Root biomass was nearly equal to shoot biomass at 6 MAP but had decreased on average to less than half of the shoot biomass by 18 MAP as the root growth rate decreased. By 18 MAP, the percent non-ECM had dropped to levels consistent with other studies (Jonsson et al. 1999; Jones et al. 2002).

### Effects of proximity to residual trees on seedling mycorrhizas

Seedlings 2–6 m from residual trees had a significantly larger proportion of active ECM root tips than those 16–30 m from trees. This corresponded to the significantly smaller proportion of necrotic root tips for 2–6 m seedlings, while distance from the tree did not appear to be a significant factor influencing the proportion of non-ECM root tips. The predominance of necrotic root tips in seedlings 16–30 m from residual trees could be an indication of greater than normal fine root turnover rates, and warrants further study.

We are not aware of other studies evaluating the effect of proximity to trees on seedling ECM root turnover rates. Several studies have suggested that hyphal linkages to trees and other plants can enhance mycorrhiza colonization rates (Massicotte et al. 1994; Simard et al. 1997; Hagerman et al. 1999; Kranabetter 1999; Dickie et al. 2002; Onguene and Kuyper 2002). Dickie et al. (2002) found greater EMF colonization of red oak seedlings planted near stump-sprouting chestnut oak (*Quercus montana* Willd.) trees as did Onguene and Kuyper (2002) with ectomycorrhizal *Paraberlinia* sp. seedlings in Cameroon. Hagerman et al. (1999) found that Engelmann spruce seedlings planted in the root zone near forest–clearcut edges had greater rates of mycorrhiza colonization than seedlings planted in clearcuts; however, the edge effect was prevented by mechanical site-mounding (Jones et al. 2002). Zhou et al. (1998) also found greater mycorrhiza colonization rates in red oak seedlings planted in stands with partial canopy removal in comparison to clearcuts.

On average, seedlings 2–6 m from residual trees had a greater EMF richness index than seedlings 16–30 m from residual trees, although this difference was not significant. We have previously reported that the EMF community of 2–6 m seedlings had greater estimated species richness and diver-

**Fig. 1.** Douglas-fir seedling (*a*) percent active ECM, (*b*) percent necrotic, and (*c*) EMF richness index from 6 to 27 MAP compared by treatment group (for the EMF richness index, there were no significant differences by treatment group). Values are estimated means ± 1 SE ($N$ = 3 sites). Treatment groups with different letters at each MAP are significantly different (LSD, $\alpha$ = 0.05). Seedlings were planted 2–6 or 16–30 m from residual trees. Total number of seedlings sampled: 120.

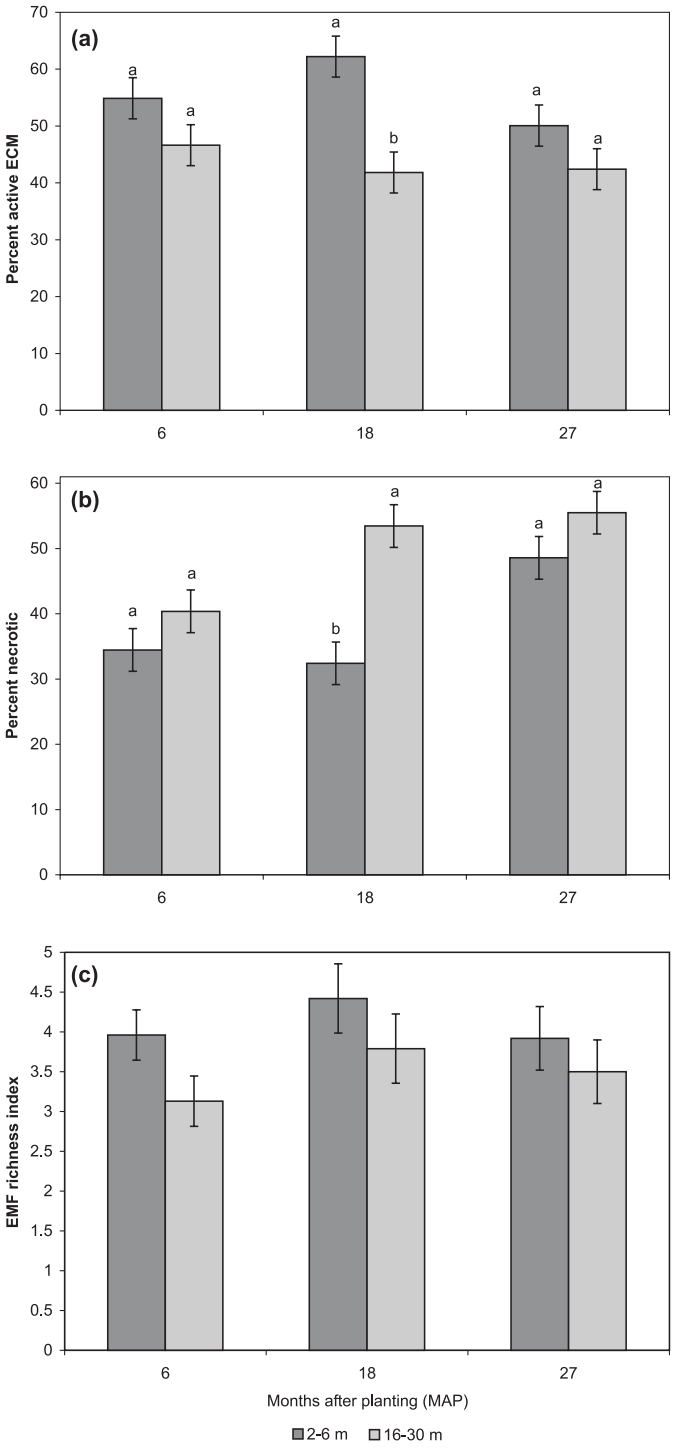


**Fig. 2.** Douglas-fir seedling (*a*) percent non-ECM and (*b*) ratio of root biomass to shoot biomass by treatment group compared from 6 to 27 MAP (Root to shoot biomass ratio significance tests were performed on log transformed data to stabilize variance; there were no significant treatment differences by LSD at each individual MAP, however, the treatment effect was significant in the repeated measures ANOVA). Values are estimated means ± 1 SE ($N$ = 3 sites). MAPs with different letters for each treatment group are significantly different (LSD, $\alpha$ = 0.05). Seedlings were planted 2–6 or 16–30 m from residual trees. Total number of seedlings sampled: 120.

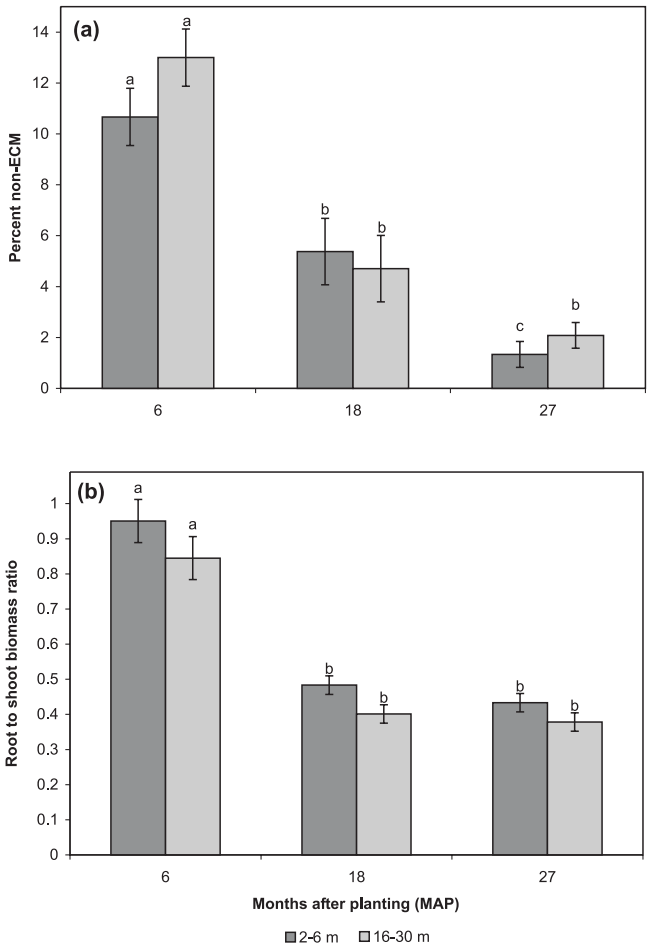


**Table 3.** Root and shoot biomass for seedlings planted 2–6 or 16–30 m from residual trees.

| Biomass | MAP | 2–6 m seedlings | 16–30 m seedlings |
|---|---|---|---|
| Root (g) | 6 | 28.0±3.5b | 29.1±3.5a |
| | 18 | 33.7±3.5b | 30.1±3.5a |
| | 27 | 41.1±3.5a | 34.3±3.5a |
| Shoot (g) | 6 | 30.9±2.4c[a] | 37.8±2.4b |
| | 18 | 71.9±8.1b | 77.1±8.1a |
| | 27 | 97.2±9.8a | 95.3±9.8a |

**Note:** Values are estimated means ± 1 SE (of seedling averages at $N$ = 3 sites). Means for MAPs with different letters are significantly different by LSD ($\alpha$ = 0.05). MAP, months after planting.

[a]Biomass estimated from seedling volume measurements.

sity (assessed by Simpson's (1/D) diversity index) in comparison with 16–30 m seedlings (Cline et al. 2005).

Several studies have demonstrated enhanced seedling mycorrhiza diversity due to proximity to trees (Deacon and Donaldson 1983; Kranabetter and Wylie 1998; Hagerman et al. 1999; Kranabetter 1999). The observed EMF taxon richness for 2–6 m seedlings in the present study (4.1) was similar to the 4.3 types per seedling reported by Simard (1995) for Douglas-fir seedlings growing under Douglas-fir trees, while seedlings prevented from hyphal linkages to outside trees by trenching had only 2.5 types per seedling. This was similar to the 2.0 EMF taxa per seedling we observed for seedlings potted in field soils from our sites and grown outside the University of Washington glasshouse (Cline 2004).

### Seedling biomass

We did not detect any significant reduction in shoot biomass for seedlings 2–6 m from residual trees, despite the potential for shading or other competitive interactions with the mature tree. Nevertheless, the significantly greater root to shoot ratios for seedlings 2–6 m from residual trees may be an initial indicator of potential future reductions in shoot growth for this treatment group. We have previously reported that at 64 MAP, after five growing seasons, seedlings 16–30 m from residual trees had a mean stem volume more than twice that of seedlings 2–6 m from residual trees, although the sample size was small and the difference was strongly site-dependent (Cline 2004). In the present study, significant treatment effects on shoot biomass might have been detected if the study had been of longer duration.

An association between higher mycorrhiza colonization levels and shifts in resource allocation to roots rather than shoots has been observed for axenically grown inoculated gray birch (*Betula populifolia* Marshall) seedlings (Baxter and Dighton 2001), for field grown red oak seedlings, especially when shaded (Zhou et al. 1998), and for subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.) and lodgepole pine seedlings naturally regenerating after wildfire (Miller et al. 1998). In contrast, field grown valley oak (*Quercus lobata* Née) seedlings had increased shoot growth and decreased root growth after soil transfers, which increased mycorrhiza infection and mycorrhiza diversity (Berman and Bledsoe 1998). Interpretation of these patterns may be complicated by the influence of other factors such as differing levels of above- and below-ground competition in the different studies.

### Conclusions

Seedlings planted in post-harvest stands are affected in various ways by proximity to residual trees. Seedlings 2–6 m from trees sustained a greater proportion of active mycorrhizal roots, which could impact their response to future fluctuations of environmental conditions. While mycorrhiza abundance and diversity may come at the cost of shifting resources from shoot growth to root growth, 2–6 m seedlings showed no decrease in shoot biomass compared to seedlings 16–30 m from residual trees over the first 27 months after planting. The long-term costs and benefits of proximity to residual trees for Douglas-fir regeneration can be established only through extending the study period beyond the scope of this project. Nevertheless, early seedling responses support residual forestry as a viable alternative to clear-cutting in managed Douglas-fir forests.

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